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Prognostic utility of HPV specific testing in addition to p16 immunohistochemistry in oropharyngeal squamous cell carcinoma

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We read with interest the paper by Nauta et al [1] since it relates to our own experience. The authors described a sub-cohort of oropharyngeal squamous cell carcinomas (OpSCCs) that were p16 positive (+) but HPV DNA negative (-) demonstrating poorer overall survival (OS) compared to p16+/HPVDNA+ individuals. We too have identified p16+/HPVDNA- cases within a UK population, a country with a higher prevalence of p16+ OpSCC than the Netherlands.

In our study, 238 OpSCC specimens from a single centre were assessed using p16 immunohistochemistry (CINtec®, Ventana Medical Systems). 153 cases (64.3%) were p16+ and were subsequently tested for high-risk (HR) HPV DNA using in-situ hybridisation (ISH, Ventana Medical Systems). Of the p16+ cases, 127 (83.0%) were HRHPV DNAISH+ whilst 26 (17.0%) were HRHPV DNAISH-. The p16+/HPVDNA- rate in our cohort was higher than that reported by Nauta et al (17.0% vs 12.4%) which may be explained by differences in sensitivity and specificity between DNAISH and PCR [2].

Similar to Nauta et al, our p16+/HPVDNA- cases demonstrated improved OS compared to p16- individuals but showed poorer survival than p16+/HPVDNA+ patients ($p < 0.001$; Figure 1A). Our p16+/HPVDNA- patients demonstrated better OS compared to the Dutch cohort and this too may be explained by the suboptimal sensitivity of DNAISH [2].

Since p16+/HPVDNAISH- cases could be due to either false positive p16 or false negative DNAISH, we used mRNAISH (RNAScope, ACDBio) as a third-tier test to resolve HPV status in p16+/HPVDNA- cases. This enabled us to further classify 15

(57.7%) and 11 (42.3%) cases as positive and negative for HRHPV, respectively. Interestingly, the OS of p16+/HPVDNAISH-/HPVRNAISH+ was similar to that of p16+/HPVDNAISH+ (Figure 1B), likely reflecting the greater sensitivity of mRNAISH [3]. Conversely, there was no significant difference in OS between p16+/HPVDNAISH-/HPVRNAISH- and p16- OpSCCs ($p=0.626$, Figure 1C). Our data therefore support the findings of Nauta et al in demonstrating poorer survival outcomes of p16+/HPVDNA- compared to p16+/HPVDNA+ OpSCC. We agree that it is important *'to perform additional HPV DNA testing for predicting prognosis and when considering treatment de-intensification'* [1].

Several authors have recently detailed the performance and utility of various laboratory HPV testing options in OpSCC [3, 4]. In this context it is important to note that inaccurate assessment of HPV status presents a hazard to patients enrolled in de-intensification trials by inappropriately assigning individuals to dose-reduction arms. In addition to exposing patients to sub-therapeutic regimes, the lack of specificity of p16 indicates that results of such de-intensification trials need to be interpreted with caution. To avoid such complications, we recommend a tiered algorithm utilising p16 immunohistochemistry, HR-HPV DNAISH and HR-HPV mRNAISH where the last provides an alternative to PCR in p16+/DNAISH- cases.

It may be tempting to recommend mRNAISH as a single assay for HPV classification in OpSCCs, but a tiered algorithm with two or more strata almost always demonstrates greater utility in avoiding inaccurate results. Furthermore, the cost implications and lack of widespread availability restricts the routine use of mRNAISH the as the second-tier test in p16+ OpSCCs [5]. We therefore suggest that

mRNAISH be reserved as a third-tier test for OpSCCs that are p16+/DNAISH-. Patients with p16+ tumours that show negativity with both DNAISH and mRNAISH could represent a separate sub-group with a different prognosis altogether, and as such should be considered for exclusion from de-intensification trials.

References

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Figure legend

Figure 1. A. Kaplan-Meier curve for overall survival according to p16+/DNAISH+ (blue line), p16+/DNAISH- (green line), p16- (red line) status (Log rank test; $\chi^2 = 48.83$; df = 2; $p < 0.001$). Significant difference in five-year overall survival was seen

between p16+/DNA ISH- (green line) and p16- (red line) cases ($p = 0.023$), as well as between p16+/DNA ISH- (green line) and p16+/DNA ISH+ (blue line) cases ($p = 0.022$). **B.** Kaplan-Meier curve for overall survival according to p16+/DNAISH+ (blue line) and p16+/DNAISH-/RNAISH+ (magenta line) status. (Log rank test; $\chi^2 = 0.12$; $df = 1$; $p = 0.727$). **C.** Kaplan-Meier curve for overall survival according to p16+/DNAISH-/RNAISH- (grey line) and p16- (red line) status. (Log rank test; $\chi^2 = 0.29$; $df = 1$; $p = 0.626$).

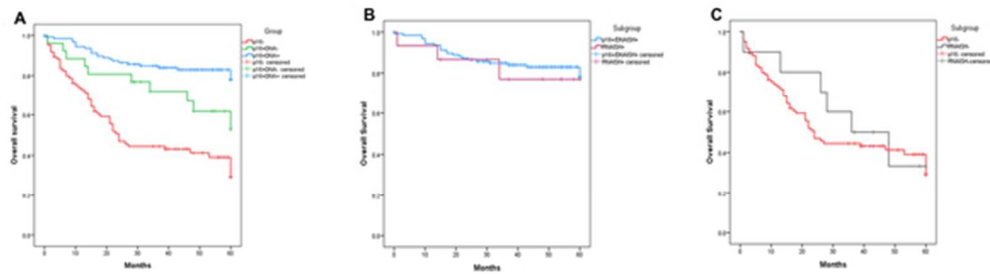


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